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Several diseases have been linked to the insertion of human LINE-1 retrotransposons (L1Hs) into structural genes. Recently, the element has been shown to be expressed in a variety of adult and pediatric germ cell cancers, leading to speculation that L1Hs-induced insertion mutation may play a role in the etiology of some neoplasias.

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Expression of LINE-1 Retrotransposons in Human Breast Cancer

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Background. **Several diseases have been linked to the insertion of human LINE-1 retrotransposons (L1Hs) into structural genes. Recently, the element has been shown to be expressed in a variety of adult and pediatric germ cell cancers, leading to speculation that L1Hs-induced insertion mutations may play a role in the etiology of some neoplasias.**

Methods. **An L1Hs-encoded protein (p40) was assayed in breast cancer cell lines by Western blotting and in solid tumors by immunohistochemical staining and Western blotting.**

Results. **L1Hs retrotransposons are expressed in a significant number of human breast cancers: expression was detected in 7 of 8 malignant cell lines and in 9 of 12 primary infiltrating ductal carcinomas. No expression was detected in two nonmalignant breast epithelial cell lines, five malignant B- or T-cell lines, tissue from a normal breast, a primary breast sarcoma, or a primary medullary carcinoma of the breast.**

Conclusions. **These results raise the possibility that L1Hs expression may contribute to the origin or progression of some breast cancers. *Cancer* 1994; 73:2333–36.**

Key words: breast cancer, retrotransposon, gene expression, immunohistochemistry.

The human genome contains a class of middle repetitive DNA known as long interspersed elements (LINE-1 Homo sapiens [L1Hs]).[1] Most L1Hs elements are truncated and rearranged pseudogenes, but a small number of transcriptionally active elements are also known to exist,[2] and recently, one of these active elements has been identified and cloned.[3] L1Hs elements have been amplified through retrotransposition: L1Hs RNAs are copied into cDNA by an L1Hs-encoded reverse transcriptase and subsequently integrated into the genome at random positions.[1,2,4] This process can result in mutations when L1Hs cDNA enter structural or regulatory genes, and human diseases that involve the integration of L1Hs elements are known.[5]

Previous results with cell lines suggested that L1Hs expression might occur in some germ cell cancers: an L1Hs-encoded protein (p40) was detected by Western blotting in the teratocarcinoma cell lines NTera2D1 and 2102Ep and in the choriocarcinoma line JEG-3.[6] Confirming the results with cell lines, high levels of L1Hs expression have been detected in primary and metastatic germ cell tumors by immunohistochemistry, leading to the speculation that L1Hs is involved in some germ cell neoplasias.[7,8]

The finding of an L1Hs element inserted de novo into a *myc* allele in a primary breast carcinoma[9] and into the APC tumor suppressor gene in a colorectal cancer[10] suggests that L1Hs elements are active in cancers other than germ cell cancers. However, these were single, isolated cases and they provide no information concerning the incidence of L1Hs expression in non–germ cell cancers. With this in mind, we have examined a number of breast cancers for L1Hs expression. In the current study, we found that L1Hs is expressed in a significant proportion of solid breast tumors and in cell lines derived from breast tumors.

Materials and Methods

Breast cell lines were obtained from the American Type Culture Collection (Rockville, Maryland) and B- and T-cell lines from G. Heidecker (National Cancer Institute/National Institutes of Health, Bethesda, MD) or the

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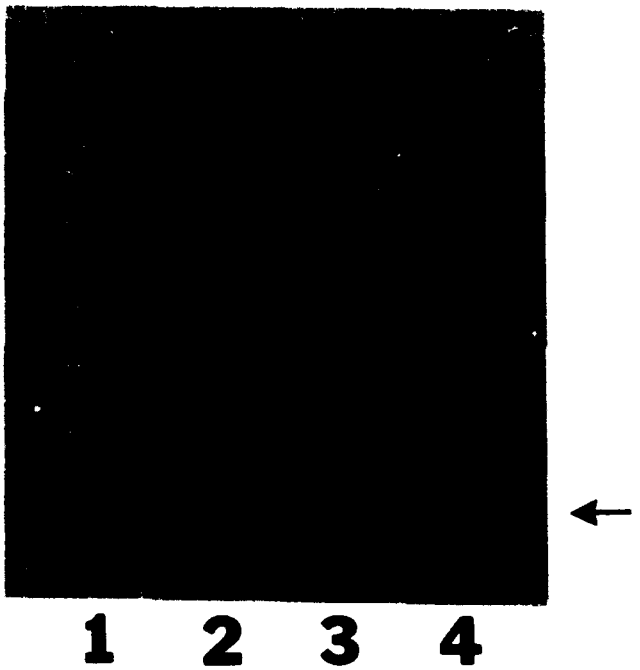

Figure 1. Western blot analysis of tumor cell lines. Lanes 1, 2, and 4 contain total cell extracts from SK-BR-3, T47D, and Ntera2D1, respectively. Lane 3 has markers. The arrow identifies the p40 protein. Bands above the p40 band in the breast tumor lines are not related to the p40 protein, because they are also present using the detection system alone.

American Type Culture Collection. Antibody AH40.1 was prepared against a TrpE-p40 fusion protein as described previously,[6] and control experiments demonstrated that AH40.1 is specific for the p40 protein.[6-8] Gel electrophoresis was performed by standard methods, and proteins (circa 30 μg/sample) were blotted onto Immobilon-P membranes (Millipore Corp., Bedford, MA). Primary antibody was used routinely at a 1:1000 or 1:2000 dilution, and staining was done with the Vectastain ABC Kit (Vector Labs, Burlingame, CA) or with the enhanced chemiluminescense method (Amersham, Arlington Heights, IL). Immunohistochemical staining of frozen tissue sections was done by a standard peroxidase–antiperoxidase method after first fixing the section with ethanol acetic acid (95:5). Primary antibody (sample 4577) was used routinely at a 1:100 dilution on frozen sections.

Results

L1Hs Expression in Cell Lines

The AH40.1 antibody recognizes the product of the first open reading frame of the L1Hs element, the p40 protein.[6] Western blot analysis of various cell line extracts with AH40.1 demonstrated that seven of the lines had detectable amounts of p40 (Fig. 1, lane 1). Blots incubated with preimmune serum or with p40 antiserum that had been preabsorbed with the p40 protein showed no p40 band. Table 1 lists the lines and the relative amounts of p40 protein found in them. These relative amounts were estimated by visually comparing the p40 band present on the blot with a control, NTera2D1, which is known to express the p40 protein abundantly.[6]

Four of the cell lines examined were derived from infiltrating ductal carcinomas, and each had a characteristic amount of p40 ranging from substantial (T47D line) to undetectable (Hs857T line) (Table 1). Thus, there appears to be no correlation between p40 quantity and primary tumor histology, at least for the small number of cell lines we examined.

In addition to the breast tumor lines, we examined two nonmalignant breast epithelial lines and five malignant nonepithelial cell lines of B- or T-cell origin. All were negative for p40 (Table 1). Because none of the malignant nonepithelial tumor lines express L1Hs (Table 1), it appears unlikely that the malignant phenotype alone is sufficient to cause L1Hs expression.

L1Hs Expression in Tumors

To extend the results with the breast tumor lines, we examined 13 primary breast carcinomas by immunocytochemistry and found that 9 were immunoreactive

Table 1. p40 in Human Cell Lines

Cell line	Type	Relative amount p40
Breast		
BT-474	Infiltrating ductal	++
HBL-100	Normal (nonmalignant)	–
Hs587Bst	Normal (nonmalignant)	–
Hs587T	Infiltrating ductal	–
MCF7	Adenocarcinoma	+
MDA-MB-453	Carcinoma	+
MDA-MB-468	Adenocarcinoma	+
SK-BR-3	Adenocarcinoma	+
T47D	Infiltrating ductal	+++
ZR-75-1	Infiltrating ductal	+
Nonepithelial		
AA2	T-cell lymphoma	–
HL-60	Promyelocytic leukemia	–
Hut78	T-cell lymphoma	–
Molt-4	Lymphoblastic leukemia	–
Raji	Burkitt's lymphoma	–

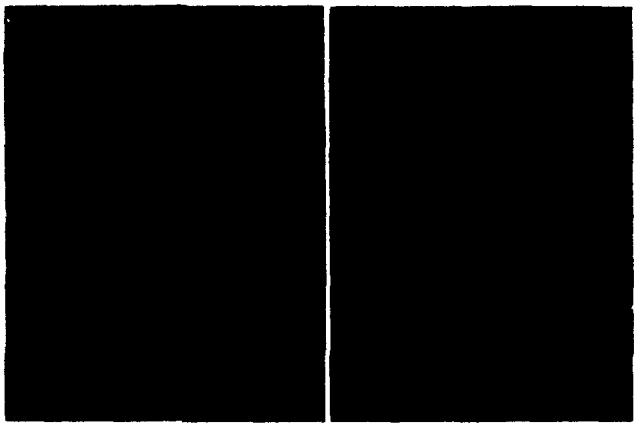

Figure 2. Immunohistochemical staining of an infiltrating ductal carcinoma (tumor N01) with AH40.1 antibody. Tumor tissue was treated with (a) the AH40.1 antibody or (b) preimmune serum and counterstained with hematoxylin (original magnification ×400).

with the AH40.1 antibody (Figs. 2 and 3). All positive cases were infiltrating ductal carcinomas (Table 2). Four breast carcinomas were unreactive with the antibody: three cases were infiltrating ductal carcinomas and one was a medullary carcinoma. Tissues from a normal, noncancerous breast and from a breast sarcoma were also unreactive (Table 2). Preimmune serum was not reactive on the positive cases, nor was p40 antiserum preabsorbed with the p40 protein in those cases that were so tested.

The nine positive cases exhibited various degrees of staining, suggesting that L1Hs expression is variable from tumor to tumor. The results with primary tumors are therefore similar to the results with breast tumor cell lines that also show variability in p40 expression (Table 1).

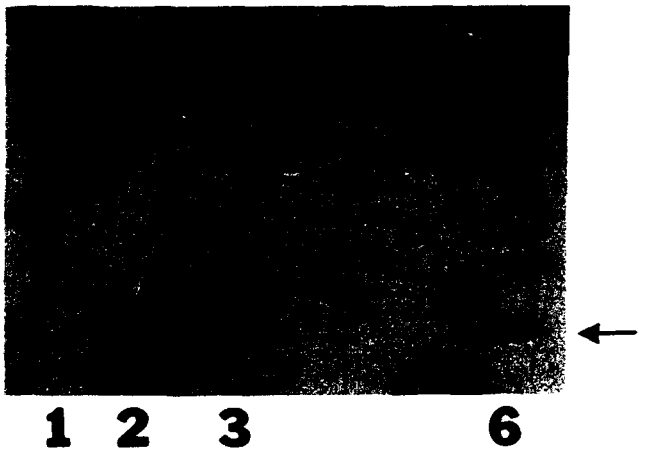

Figure 3. Western blot analysis of tumor N01. Lane 1: markers; lane 2: Ntera2D1 cell line extract; lane 3: 1/5 the protein present in lane 2; lanes 4 and 5 are blank; lane 6: N01 tumor extract. The arrow identifies the p40 protein.

Table 2. p40 in Primary Breast Tumors

Tissue	No.	p40+	p40−
Infiltrating ductal	12	9	3
Medullary carcinoma	1	—	1
Sarcoma	1	—	1
Normal breast	1	—	1

To verify further that staining in the primary tumors was due to p40, we extracted protein from a small amount of a tumor designated N01 and performed a Western blot analysis. This tumor was one of the most reactive with the AH40.1 antibody (Fig. 2a), and the results verified that p40 is expressed in tumor N01 (Fig. 3). The sharpness of the p40 band in tumor N01 is interesting, because in vitro translation of cloned L1Hs cDNA demonstrated that different L1Hs family members encode p40 proteins with unique mobilities on acrylamide gels.[6] Thus, it is possible that this solid tumor has only one active class/type of L1Hs element.

Discussion

Breast cancer is one of the most common cancers and is postulated to arise by multistep processes involving the activation of oncogenes and the loss of tumor suppressor genes.[11,12] Although a number of these steps have been elucidated, many of the events involved in the initiation and maintenance of this cancer are unknown.[12]

Could L1Hs be involved in the initiation or progression of breast cancer or other epithelial cancers? The evidence that it might be consists of the following: (1) a large proportion of certain cancers of epithelial origin contain the L1Hs-encoded p40 protein, (2) normal epithelial cells have no detectable p40, and (3) nonepithelial malignant cells have no detectable p40. The first point demonstrates that L1Hs expression is not a rare event in some cancers (69% for primary breast carcinomas and 88% for breast carcinoma cell lines). The second and third points indicate that L1Hs is not generally expressed in human tissues, be they normal or malignant.

There are several characteristics of the L1Hs element that suggest its potential as an oncogenic agent. One possibility involves the L1Hs-encoded reverse transcriptase. This enzyme may copy cellular RNA (LINEs, SINEs, mRNA) into cDNA, which are subsequently integrated into the genome, leading to the inactivation of important regulatory genes. The inactivation of the APC tumor suppressor gene by de novo insertion of an L1Hs element in a colorectal cancer appears to be such an event.[10]

Although insertion mutagenesis is the most obvious

mechanism by which L1Hs might alter cellular metabolism, several additional mechanisms are also possible. Although L1Hs expression has not been found in normal tissues, many cells appear to contain a DNA-binding protein that may be involved in L1Hs transcription.[13] This protein might interact with the L1Hs internal promoter[14] in neoplastic cells, leading to read-through transcription and activation of genes downstream of the L1Hs element. In addition, the L1Hs-encoded p40 protein has a leucine zipper motif,[15] suggesting a possible interaction with other cellular proteins. Such interactions, at inappropriate times, might lead to the disruption of important cellular functions.

The preceding possibilities are only speculation. However, all have been documented with other oncogenes and oncogenic agents.[16] Some major facets of L1Hs biology to be answered by future research include the influence of abundant p40 expression on cellular metabolism and determination of the ratio between the frequency of L1Hs expression and the frequency of cDNA insertions into important genes.

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